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United States Department of Agriculture–Agricultural Research Service research programs in biological control of plant diseases†‡

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Abstract: A number of USDA–ARS programs directed at overcoming impediments to the use of biocontrol agents on a commercial scale are described. These include improvements in screening techniques, taxonomic studies to identify beneficial strains more precisely, and studies on various aspects of the large-scale production of biocontrol agents. Another broad area of studies covers the ecological aspects of biocontrol agents—their interaction with the pathogen, with the plant and with other aspects of the environmental complex. Examples of these studies are given and their relevance to the further development and expansion of biocontrol agents is discussed.
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1 INTRODUCTION

Biological control of plant diseases can be defined as the reduction of inoculum or disease-causing activity of a plant pathogen through the use of organisms other than man.[1,2] Reducing chemical inputs in agriculture is the major impetus for developing biological controls for the suppression of plant pathogens. Concerns about the impact of agricultural chemicals and pesticides on human health and the environment require that chemical inputs in agriculture be reduced.[3] In addition to substantial reductions in the use of chemical pesticides, the incorporation of appropriate biological controls for the management of plant pathogens may maintain greater biological balance and diversity in the environment, lead to more sustainable long-term agricultural production practices, and, in some cases, achieve better disease control than with current disease control methods.[3]

The potential benefits of biological control to agriculture have generated tremendous research interest since the 1960s.[4] The disease-control activity of numerous potential microbial biological control strains under laboratory conditions has been documented in research publications. However, the transition of biological control in the laboratory to successful biological control under commercial agricultural conditions has been slow.[3] Only a small proportion of microbial biocontrol agents studied in the laboratory are currently registered and sold in the USA. Many factors are responsible for the poor transition of biocontrol agents from laboratory test strains to commercial products. These include: (1) inappropriate screening processes for the isolation of potential biocontrol agents, (2) biomass stabilization or formulation difficulties, (3) inconsistent product performance due to insufficient knowledge of the ecology of the microbial antagonist, (4) insufficient knowledge of the target pathogen and associated microflora, (5) disease control is not as complete as with chemicals, (6) cost-prohibitive registration processes, (7) lack of patent protection of a potential product formulation or active ingredient, and (8) unfavorable economics of biomass production or market size.[3,5–7] The Agricultural Research Service (ARS) has several active research programs directed at overcoming impediments to commercialization of biocontrol agents. Recent research progress from several ARS laboratories is highlighted below.

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2 SCREENING FOR BIOLOGICAL CONTROL AGENTS

2.1 Mass screening of potential biocontrol agents for disease suppression

Poor commercialization of biocontrol agents is due, in part, to poorly designed screening strategies for the selection of biocontrol agents. A poorly conceived screening strategy can be a costly mistake since the selection of candidate microbial organisms comes at the beginning of a long development process.[5] When possible, disease suppression assays that closely mimic the environmental conditions of commercial production conditions should be performed with genetically diverse isolates of potential biocontrol agents. In our own work, screening was performed in fields artificially infested with the take-all pathogen.[8] Direct screening in the field was less time-consuming than similar studies performed in the greenhouse or growth chamber, and the results were more applicable to actual commercial production conditions. We isolated candidate bacterial strains on various media and identified them using fatty acid methyl ester profiles. Dendrograms were subsequently generated to select bacterial strains with maximum genetic diversity for incorporation into our disease suppression screens. Identification and determination of the genetic-relatedness of the bacterial strains incorporated into the screen allowed us to increase the effectiveness of our screen by maximizing the incorporation of unrelated strains. Other work indicates that soil effects on microbial communities in the spermosphere and rhizosphere are greater than plant effects (Buyer JS, pers comm, and Reference 9). This suggests that biocontrol assays in soil should be performed in a variety of soil types to assess the ability of candidate biocontrol strains to establish and suppress disease in various microbial communities as well as under a variety of different soil conditions. Direct screening under commercial conditions is sometimes impractical and expensive in terms of labor, space and material. Bowers *et al* (pers comm) have developed a leaf disk assay for screening potential biocontrol strains for suppression of *Phytophthora megakarya* Brasier & Griffin on cacao. Cacao pods are harvested from trees grown as an understory crop making direct screen methods impractical. This leaf disk method provides the convenience and cost effectiveness of *in vitro* methods while providing a plant surface on which biological control interactions can be tested.

2.2 Considerations for selecting biocontrol agents for further development

Work by Larkin and Fravel[10] indicates the need for rigorous testing to determine conditions required for potential biocontrol agents to be effective. These authors worked with three non-pathogenic *Fusarium* isolates that were previously shown to have comparable disease suppression of Fusarium wilt on tomato in inundative greenhouse assays.[11] It was subsequently shown that these three isolates differed with regard to mechanisms of action, dose-response relationships and disease suppression efficacy.[10] Isolate CS-20 was effective at low inoculum densities and at all pathogen densities. In contrast, biological control by isolate CS-1 was not effective at high pathogen densities, while isolate Fo47 was effective at inoculum densities ten to one hundred times that of the pathogen. Isolate CS-20 was selected for further study.[10]

Recent work by Schisler *et al*[12] underscores the need for the use of multiple cultivars and pathogen isolates during the selection of biocontrol agents for further development. Five bacterial antagonists demonstrated to suppress Fusarium dry rot on stored potato tubers were assayed for effectiveness against ten isolates of the pathogen, *Gibberella pulicaris* (Fr) Sacc. Four of the five antagonists had relative disease-suppression rankings that varied from first to last depending on the pathogen isolate used in the assay. The cultivar of potato used also affected relative antagonist performance.[12] Other work from this ARS group indicates that the process of choosing the best candidate biological control agent should include amenability of the candidate microbe to scale up commercial production conditions.[5] The microbe must have favorable growth kinetics and biomass yield in liquid culture.

2.3 Selection of biocontrol agents with enhanced performance

Gu and Mazzola used repeated subculturing of *Pseudomonas putida* (Trev) Mig 2C8 to enhance the biocontrol ability of this strain.[13] *Pseudomonas putida* 2C8 was isolated from the rhizosphere of apple and selected for further study due to its ability to suppress Rhizoctonia root rot of apple caused by *Rhizoctonia solani* Kuhn and its ability to enhance growth of apple in orchard replant soils.[14,15] Derivatives of strain 2C8, that had been repeatedly subcultured onto soil agar amended with a diluted suspension of King's medium B exhibited enhanced stress resistance and were not reduced in antifungal properties. These derivative strains were also enhanced in colonization of apple rhizosphere and suppression of apple root infection by *R solani*.[13] These results suggest that exposure to certain conditions *in vitro* can be used to select biocontrol strains with enhanced disease suppression.

3 TAXONOMY OF BIOCONTROL AGENTS AND PLANT PATHOGENS

Morphological and molecular approaches have been used by ARS scientists and collaborators in phylogenetic and taxonomic studies of genera that contain microbial biological control agents such as *Fusarium*, *Pythium* and *Trichoderma*.[16–23] This information is important for distinguishing beneficial microbes from plant pathogens and for patenting purposes. For example, taxonomic studies of the genus *Trichoderma* have allowed important beneficial *Trichoderma* strains to be distinguished from the pathogenic *Trichoderma*

strains that cause green mold on cultivated mushrooms (Samuels *et al*, pers comm).

4 FERMENTATION AND FORMULATIONS OF BIOCONTROL AGENTS

Barriers to the commercial use of biocontrol agents include the lack of liquid-culture and formulation technologies needed to optimize mass production of these microbes.[24] For yeast and bacterial strains, commercial production of biomass for biocontrol preparations generally requires liquid culture production in large, industrial scale fermenters. Disease suppression efficacy of biocontrol strains varies with the medium used in fermentation for production of biomass, and strains can vary with regard to their amenability to liquid culture fermentation.[5] For example, the relative biocontrol efficacy of five antagonists used to suppress Fusarium dry rot of potato varied with the medium used to produce the preparations of antagonist.[5] In other studies it has been shown that inoculum preparation influences establishment of bacterial antagonists of *Erwinia amylovora* (Burrill) Winslow *et al* on apple and pear blossoms. In three of five trials, bacteria applied as suspensions of lyophilized cells were recovered from a greater proportion of blossoms than bacteria taken directly from culture media.[25]

Other studies indicate that culture physiological state, metabolites produced during fermentation and the biocontrol formulation can affect the long-term viability of biocontrol preparations, phytotoxicity of these preparations and efficacy of disease suppression.[24] Significant germination losses were detected in wheat seed treatments containing the biocontrol agent *Pseudomonas fluorescens* Mig 2-79 in growth chamber studies. These reductions in seed germination were attributed to metabolites produced by strain 2-79 during fermentation that were encapsulated in the seed treatment.[24] Metabolites accumulated during fermentation can have a direct impact on the subsequent quality of the biocontrol preparation in terms of storage stability, seed compatibility and disease suppression efficacy.[24,26] Work has also been directed at determining the metabolites produced by strain 2-79 during fermentation on particular media and manipulating fermentation to enhance performance of biological control preparations by altering production of these metabolites.[27]

ARS groups have been investigating the application of biocontrol agents in formulations in combination with compounds that have antimicrobial properties.[28–33] For example, coatings of apples containing the biocontrol yeast *Candida saitoana* and glycolchitosan were more effective in controlling decay in several cultivars of apples than either *C saitoana* or glycolchitosan applied alone.[30] In other studies, it was shown that application of shellac and other natural products in fruit coatings supported survival of the yeast *C oleophila* Montrouchet for biological control of post-harvest decay of grapefruit.[34]

5 ECOLOGICAL INTERACTIONS AND BIOLOGICAL CONTROL

Disease suppression by biocontrol agents is the result of the complex ecology among the plant, the biocontrol agent, the microbial community on and adjacent to the plant, the pathogen and the physical environment.[35] These ecological interactions are highly complex and poorly understood even in simplified laboratory model systems. Understanding these interactions is key to determining the conditions and requirements necessary for biological control processes to function effectively.[3]

5.1 Interactions of the biocontrol agent with the pathogen

5.1.1 Metabolic inhibitors produced by bacterial biocontrol agents

Metabolic inhibitors produced by biocontrol agents can function in suppression of disease caused by plant pathogens. These inhibitors, such as antibiotics, can suppress the activity of the pathogen or destroy pathogen propagules.[3] Several ARS groups have active research programs directed at understanding the nature of antibiotics and other metabolic inhibitors in disease suppression. Seminal work in this area was done by Thomashow *et al*[36,37] Studies with near-isogenic mutants of the biocontrol bacteria *P fluorescens* 2-79 and *P aureofaciens* 30-84, that were deficient in production of phenazine antibiotics, demonstrated that these phenazine antibiotics were produced in wheat rhizosphere and that these antibiotic compounds play a major role in suppression of the take-all pathogen, *Gaeumannomyces graminis var tritici* J Walker (Ggt), on wheat by these strains.[36–38] In addition, studies with these phenazine-deficient mutants clearly demonstrated a role for these compounds in the long-term survival of these strains in the rhizosphere.[39]

Recent work by Thomashow and co-workers[40] was directed at organizational and functional analysis of the genetic locus for phenazine-1-carboxylic acid biosynthesis in *P fluorescens* 2-79. A seven gene locus (*phzABCDEFG*) located on a 6.8-kb DNA fragment was sequenced. Other results indicated that different species of fluorescent pseudomonads have similar genetic systems that confer the ability to produce phenazine-1-carboxylic acid.[40] Work by this group with *P aureofaciens* 30-84 identified a novel gene, *phzO*, located downstream from the core phenazine biosynthesis operon, that encodes a 44-kDa aromatic monooxygenase that hydroxylates phenazine-1-carboxylate to produce 2-hydroxy-phenazine.[41] Knowledge of genes responsible for phenazine product specificity could reveal ways to manipulate organisms to produce novel or multiple phenazines.[41] This group is doing similar studies with 2,4-diacetylphloroglucinol (DAPG) biosynthetic pathways in certain *Pseudomonas* strains.[42]

5.1.2 Global regulators of antibiotic production

Work by several ARS laboratories demonstrated that *P fluorescens* Pf-5 suppresses damping-off caused by *Pythium ultimum* Trow on cotton and cucumber and by *R solani* on cotton.[43–45] This bacterial strain also inhibits ascocarp formation by *Pyrenophora tritici-repentis* (Died) Drechsler on wheat straw debris.[46] *Pseudomonas fluorescens* Pf-5 produces the polyketide antibiotics pyoluteorin and DAPG,[43,47] the antibiotic pyrrolnitrin,[45] and hydrogen cyanide.[44] Work by Loper and co-workers characterized the pyoluteorin biosynthetic gene cluster of Pf-5.[48,49] Gene fusion studies with a promoterless *inaZ* reporter indicated that genes for pyoluteorin biosynthesis are expressed in cucumber and cotton spermosphere.[50]

Recent work by Loper and co-workers indicates that production of antibiotics by *P fluorescens* Pf-5 is modulated by at least four global regulatory genes that have pleiotropic effects on this bacterium. *gacA* and *gacS* are required for production of pyoluteorin, pyrrolnitrin, and DAPG by Pf-5. This two-component regulatory system is also important for Pf-5 to tolerate oxidative stress.[51] The sigma factor, σ^s, encoded by *rpoS*, modulates antibiotic production by Pf-5.[51] A Pf-5 mutant, with a transposon insertion in *rpoS*, overproduces the antibiotics pyoluteorin and DAPG, but does not produce pyrrolnitrin. This *rpoS* mutant was superior to wildtype Pf-5 in suppression of *P ultimum* on cucumber.[52]

Characterization of *rpoS*, *gacA*, and *gacS* by Loper and co-workers has provided insight into the complexities of antibiotic production and disease suppression by *P. fluorescens* Pf-5. Recent work by this ARS group has identified a fourth global regulatory gene, *lon*, involved in modulating antibiotic production and in stress responses by Pf-5.[53] A transposon mutant of Pf-5 was isolated that overproduced pyoluteorin. Using a molecular approach, the gene inactivated by the transposon insertion was cloned, sequenced and identified as *lon*. Using transcriptional gene fusions with the ice nucleation reporter gene the influence of *lon* on genes involved in pyoluteorin synthesis was assessed. *lon* is a global regulator that negatively influences pyoluteorin production. Other work reported with this study indicates that *lon* is also involved in responses to UV irradiation and heat shock stresses. Understanding the impact of various environmental stresses on global regulators and antibiotic production may allow manipulation of antagonist strains to improve biological control performance.[53]

5.1.3 Metabolic inhibitors produced by fungal biocontrol agents

ARS scientists have also investigated antibiotic-producing fungal biocontrol strains. Different strains of the fungal biocontrol agent *Trichoderma virens* (Miller, Gidders & Foster) produce the antibiotics gliotoxin and gliovirin.[54,55] Production of gliotoxin was correlated with biological control efficacy of *T virens* strain GL-21, the active component of the commercial product Soil Gard™. Mutants of *T virens* GL-21 that do not produce gliotoxin are reduced in their ability to control damping-off caused by *P ultimum* and quantities of gliotoxin in soils colonized by *T virens* were correlated with disease suppression.[56,57] Work with *Talaromyces flavus* Stolk & Samson, a biocontrol fungus used to suppress *Verticillium dahliae Kleb* on eggplant, has demonstrated that antibiosis of *V dahliae* is due to the evolution of hydrogen peroxide in soil.[58–62] Hydrogen peroxide, produced in a reaction catalyzed by the *T flavus* enzyme glucose oxidase, was sufficient to kill microsclerotia of *V dahliae* in the rhizosphere. In addition, a variant strain of *T flavus* that was reduced in glucose oxidase production was also reduced in suppression of Verticillium wilt of eggplant in natural soil.[59] More recently, antibiotics have been characterized from culture extracts of fungi that colonize sclerotia of fungi such as *Aspergillus flavus* Link.[63–65] The sclerotia of *A flavus* are an important source of inoculum for this aflatoxin-producing fungus.[66]

5.1.4 Competition for nutrients and disease suppression

Disease suppression can be the manifestation of competition for nutrients, where the biological control agent deprives the plant pathogen of nutrients required by the pathogen for infection of the plant or for pathogen survival. Perhaps the best-studied example of this is competition for iron between the biocontrol agent and plant pathogen in the rhizosphere and on plant surfaces. Iron is limiting at neutral pH and above due to the formation of highly insoluble ferric oxyhydroxides. Microorganisms excrete siderophores to aid in satisfying iron nutritional requirements. Siderophores bind soluble iron, allowing the biocontrol agent to compete effectively with the pathogen for iron. One ARS group has an active research program investigating the role of siderophore production by biocontrol agents in disease suppression. Recent developments from this group include characterization of siderophores produced by biocontrol agents,[67] the development of a reporter gene system to evaluate iron availability in the rhizosphere to *P fluorescens* Pf-5,[68] and that biocontrol bacteria in the rhizosphere can utilize heterologous siderophores.[69]

5.2 Analysis and manipulation of plant associated microbial communities for disease suppression

5.2.1 Characterization of suppressive soils

Weller and co-workers are in the initial stages of characterizing soils naturally suppressive to the take-all pathogen Ggt. It is widely held that take-all decline is based on microbial interactions between Ggt and wheat-root-associated microbes.[70] Of these wheat-root-associated microbes, much attention has been given to phenazine- and DAPG-producing *Pseudomonas* strains. Work by Weller and co-workers has

shown that: (1) the DAPG-producing strains were key components of suppressive soils in Washington state, (2) suppression in the suppressive soils was lost when DAPG-producing *Pseudomonas* strains were eliminated from these soils, and (3) suppression in these soils was regained by adding DAPG-producing *Pseudomonas* strains back to these soils.[71] Further evidence for a major role for DAPG-producing pseudomonads comes from the detection of DAPG in wheat rhizosphere in a soil suppressive to take-all,[72] and molecular studies showing that DAPG-producing strains inactivated in DAPG production were diminished in suppression of take-all.[73–76] Characterization of microbial communities naturally suppressive to soilborne plant pathogens may provide insight into biocontrol interactions and provide a rich resource of uncharacterized microbes such as strains with superior root colonizing ability.[71,77] It was shown that DAPG-producing pseudomonads in suppressive soils were genetically diverse, and one strain, Q8r1-96, which is representative of a particular DAPG-producing genotype that is common in take-all decline in wheat rhizosphere, is a superior root colonizing strain.[77]

5.2.2 Characterization of epiphytic bacterial populations on pear blossoms

In other studies, pear blossoms were sampled at various stages of bloom for indigenous epiphytic populations of bacteria.[78] The authors found that most pear blossoms do not support detectable populations of bacteria prior to petal expansion. They speculate that it would be easier to establish bacterial biocontrol agents at early bloom stages since competition for resources will be minimized due to low populations of indigenous bacteria.

5.2.3 Manipulation of microbial communities for disease suppression

Cultivation of orchard replant soils with wheat cultivars enhanced growth of apple relative to untreated controls. Enhanced growth by apple plants was associated with reductions in root infection by *Rhizoctonia* spp and *Pythium* spp and reductions in populations of the nematode *Pratylenchus* spp. Cultivation of replant soils with wheat caused a transformation of pseudomonad populations from those dominated by *P fluorescens* biotype C and *P syringae* van Hall to those dominated by *P putida*.[79] The wheat root system supports substantial populations of strains with the 2C8 phenotype.[79,80] Strain 2C8 has been shown to suppress Rhizoctonia root rot of apple.[13] Results from this work suggest that use of short-term wheat cropping during apple orchard renovation could be a useful management tool for suppression of apple replant disease. This may operate in part through modification of the fluorescent pseudomonad community.[79]

5.3 Interactions of the biocontrol agent with the plant

5.3.1 Induction of plant defense responses

Certain biocontrol fungi and bacteria induce responses in the host plant which result in suppression of disease. Howell *et al*[81] reported that seed treatments of cotton with *T virens* resulted in terpenoid synthesis and peroxidase activity in cotton roots while nontreated controls did not show this response. Terpenoid pathway intermediates desoxyhemigossypol and hemigossypol were highly active against the plant pathogen *R solani*. In addition, induction of terpenoids by strains of *Trichoderma* spp was correlated with suppression of cotton seedling disease caused by *R solani*. Strains inducing the greatest quantity of terpenoids in cotton roots had the greatest suppression of cotton seedling disease.[81]

5.3.2 Colonization of plant surfaces

The establishment and redistribution of biocontrol agents from seeds or other application media to the rhizosphere or plant tissues is thought to be essential for successful biological control in many applications.[82] A number of studies indicate that colonization of subterranean plant parts is a limiting step in biocontrol of soilborne plant pathogens,[82–84] and a number of diverse traits have been correlated with colonization of plant surfaces, including motility, chemotaxis, salt tolerance, root attachment, putrescine uptake, O-antigen side-chain of LPS and a site-specific recombinase.[85–93]

It has also been demonstrated that growth of the microbial inoculants is indispensable for efficient colonization to occur.[94–101] In order to develop microbial inoculants that are effective biocontrol agents, it is imperative that inconsistencies in the performance of inoculant strains be resolved.[1,83] There is a consensus that these inconsistencies may be due, at least in part, to an inability of the introduced strain to compete with indigenous microorganisms for nutrients and other resources, resulting in a failure to colonize the rhizosphere effectively.[94–97,102] Seed and root exudates are a complex and rich source of carbohydrates, amino acids, organic acids and other nutrients that are thought to promote the metabolic activity and growth of beneficial bacteria in the spermosphere and rhizosphere.[103,104] However, there is a significant gap in our knowledge of the role played by the metabolic (catabolic and anabolic) pathways of biocontrol bacteria and the nutrients supplied by the host plant in growth and colonization.

Strains of the potential biocontrol bacterium *Enterobacter cloacae* (Jordan) Hormaeche & Edwards have been shown to be efficient colonizers of the spermosphere and rhizosphere of a wide range of plant species.[95–97,99,105–109] Strains of *E cloacae* have also been shown to protect cucumber and other plants from damping-off caused by *P ultimum*.[99,106,109–111] We have used a mutational approach to study the role of the bacterial genes and catabolic pathways and the nutrients supplied by the host plant during

growth and colonization of seeds and roots by *E cloacae* 501R3.[95–97,101,104]

For this work, a library of mini-Tn5 Km transposon mutants of *E cloacae* 501R3 was screened for loss of growth on minimal media containing various compounds present in exudates of plant seeds and roots.[95,96] Candidate strains that lost the ability to grow on these compounds were subsequently screened in seed and root colonization assays. A number of mini-Tn*5* Km mutants of strain 501R3 were identified that had a reduced ability to colonize seeds and/or roots (Table 1). Subsequent biochemical and molecular characterization of certain mutants identified transposon insertions in *aceF, cyaA, pfkA, rpiA*, and *sdhA* (Lohrke SM and Roberts DP, unpublished).[101,104] These genes encode enzymes that play key roles in the Embden Meyerhof Parnas pathway (*aceF, pfkA*), the tricarboxylic acid pathway (*aceF, sdhA*), and the pentose phosphate pathway (*rpiA*) (Fig 1). Transposon insertions in *pfkA* and *sdhA* lead to small, but statistically significant, reductions in colonization of cucumber by the mutant strains relative to the wild-type strain, 501R3. The mutation in *rpiA* results in the loss of cucumber, sunflower, and wheat root colonization by the mutant indicating that *rpiA* and possibly the pentose phosphate pathway play a key role in colonization of roots by *E cloacae* (Lohrke SM and Roberts DP, unpublished and References 101,104).

Knowledge of genes and pathways responsible for root colonization could reveal ways to manipulate organisms to enhance root colonization and disease suppression. One approach to improving colonization is to increase expression of genes involved in the colonization process. The concept of metabolic engineering of microorganisms to increase the productivity and/or quality of microbial fermentations has attracted increased attention from industry.[112–114]

Table 1. Colonization and biocontrol phenotypes of *Enterobacter cloacae* mutants

Mutant	Growth on seeds[a]	Root colonization[a]	Biocontrol[b]	Gene
A11	No growth on cucumber and radish	Reduced on cucumber	Same	*pfkA*
A145	No growth on corn, cowpea, cucumber, sunflower, wheat	No colonization of cucumber, sunflower, wheat	Reduced	*rpiA*
C1	No growth on cucumber	Reduced on cucumber	Same	ND[c]
C7	ND[c]	Reduced on cucumber	Same	ND[c]
C11	Reduced growth on cucumber	No reduction on cucumber	Same	ND[c]
C12	ND[c]	Reduced on cucumber	Same	ND[c]
M2	Reduced growth on cucumber and pea	Reduced on cucumber	Same	*sdhA*
M43	No growth on cucumber and radish	Reduced on cucumber	Same	*aceF*
M59	No growth on cucumber and radish	Reduced on cucumber	Same	*cyaA*
M64	Reduced growth on corn and cucumber	ND[c]	Same	ND[c]

[a] Relative to wild-type strain 501R3.
[b] Biocontrol of *Pythium ultimum*-induced damping-off relative to wild-type strain 501R3.
[c] ND, not determined.

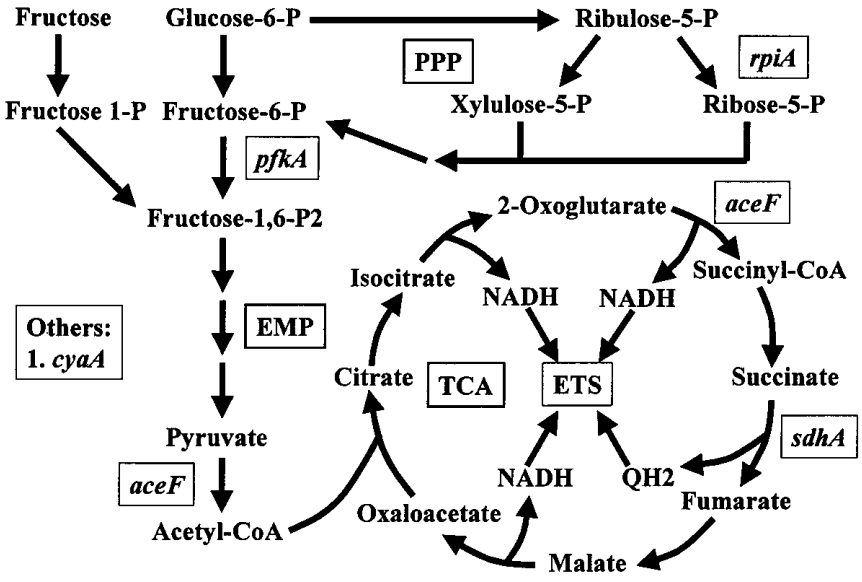

Figure 1. Identification of genes in *Enterobacter cloacae* involved in colonization and their location in central metabolism. Abbreviations: EMP, Embden Meyerhof Parnas pathway; ETS, electron transport system; PPP, pentose phosphate pathway; TCA, tricarboxylic acid cycle; *aceF*, lipoamide dehydrogenase; *cyaA*, adenylate cyclase; *pfkA*, phosphofructose kinase; *rpiA*, ribose phosphate isomerase A. *sdhA*, succinate dehydrogenase subunit A.

Within any given metabolic pathway, the metabolic flow is determined, to a large extent, by the activities of one or more key enzymatic steps. It is argued that, by increased expression of these key enzymes, metabolic flow through the pathway can be increased, which may result in increased production of the desired metabolite(s). Identification of those genes necessary for efficient colonization may provide researchers with potential targets for increased expression. This may result in an increase in the ecological fitness of the inoculant that may translate into improved colonization.

Knowledge of genes and pathways important to colonization and disease suppression may also allow spatial-temporal or nutritional separation of biocontrol strains in a biocontrol preparation containing multiple biocontrol agents. Conditions exist in agricultural fields where the crop is exposed to several plant pathogens over time. A potential solution is to apply multiple biocontrol agents targeted at different pathogens. However, combinations of strains sometimes results in poor performance by all strains in the preparation, possibly due to antagonism amongst the biocontrol strains.[115,116] For example, numbers of eggs plus second stage juveniles of the plant parasitic nematode *Meloidogyne incognita* (Kof & White) Chitwood per gram of Bell pepper root were significantly lower with treatments containing *Burkholderia cepacia* Yabuchi *et al* Burkholder, *B ambifaria*, or *T virens* than with the untreated control. Treatments containing combinations of these biocontrol microbes were not effective in suppressing populations of *M incognita*.[117] Several researchers have suggested that for improved performance to occur, strains combined in preparations must be compatible.[118–120] Spatial-temporal or nutritional separation of these biocontrol strains may increase compatibility and improve performance.

Delivering *E cloacae* to the cucumber spermosphere in the appropriate metabolic state to suppress *P ultimum* damping-off, rather than extensive root colonization by *E cloacae*, is important for success in this biocontrol interaction. A bacterium, deficient in cucumber root colonization, was effective in controlling damping-off of cucumber caused by *P ultimum*.[99] This is due primarily to the short window of vulnerability of cucumber to this disease.[99,106] Other biocontrol interactions may require extensive colonization of roots by the biocontrol agent. It may be possible to develop a temporal succession of strains by using a mutant of *E cloacae*, deficient in cucumber root colonization, to suppress *P ultimum*-induced damping-off in combination with a second biocontrol strain targeted against a pathogen that causes disease throughout the growing season. Inactivation of this gene in *E cloacae* would result in a non-persistent *E cloacae* mutant and spatial-temporal separation of the biocontrol agents in the biocontrol preparation. Also, nutritional niche separation may be possible by using strains that effectively colonize roots but use mutually exclusive classes of nutrients in the rhizosphere. In preliminary studies, it was shown that carbohydrates and amino acids are not required for suppression of *P ultimum* damping-off on cucumber. Mutants of *E cloacae* with mutations in *cyaA, pfkA, sdhA* and other genes important in catabolism of carbohydrates, amino acids and organic acids released by seeds and roots were effective in suppressing *P ultimum*-induced damping-off and were only moderately reduced in root colonization (Roberts *et al*, unpublished) (Fig 1, Table 1).

6 CONCLUSIONS

Biological control depends on the effective functioning of antagonist microorganisms within particular plant-pathogen ecosystems. Disease suppression is the result of complex interactions among the biocontrol agent, pathogen, plant and the biological and physical environment. These complex interactions are just being identified. Each antagonist has different traits whose expression results in disease suppression, and, therefore, different requirements for effective disease suppression.[3] The more that is known about the particular requirements for disease suppression in a particular biocontrol interaction the easier it will be to establish strategies that optimize biocontrol performance for that particular interaction. As indicated above, the goal of many ARS research programs is to investigate the particular requirements of biocontrol agents so that effective strategies utilizing biocontrol can be implemented.

Due to their biological nature, biological controls can be inconsistent, their performance varying with location or the growing season. It may be possible to enhance consistency and efficacy of disease suppression by using microbials for control of plant disease in conjunction with other plant health management strategies. In general, biological control does not provide broad-spectrum suppression or completely eliminate plant pathogens, may not work as fast as chemical pesticides, and may only provide partial levels of disease control.[3] ARS scientists are investigating the use of biological controls in combination with antimicrobial compounds or other compounds or with reduced levels of chemical controls,[8,28,30–33,121–123] sublethal stressing of pathogens in soils,[124,125] and the use of cover crops and compost for enhanced disease suppression.[126]

ARS has diverse and intensive research programs in biological control of plant disease. These programs are attempting to adapt biological control to field crop (ie alfalfa, corn, cotton, soybean, wheat), fruit and vegetable production.[11,12,14,30,34,37,43,59,63,70,118,127–130] There is no question that biocontrol works. Natural situations, such as suppressive soils, illustrate this. The challenge facing ARS scientists and other researchers at universities and in industry is to integrate biocontrol technology into other plant health management systems in commercial production systems for control of plant diseases.

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